



PCT/GB00/103297



INVESTOR IN PEOPLE

REC'D 28 SEP 2000 The Patent Office WPO Concept House PCT

Cardiff Road
Newport
South Wales
NP10 8QQ

GB09/03297

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

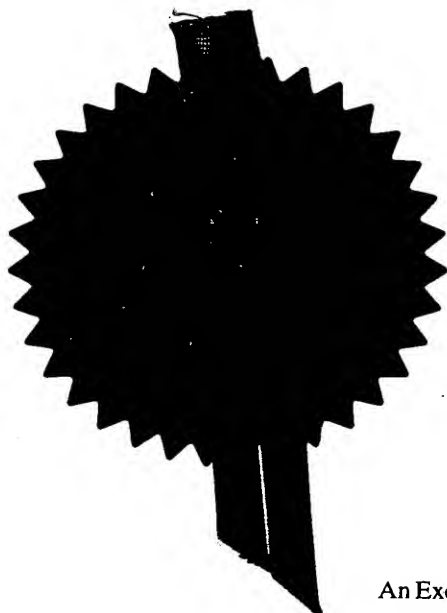
Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Andrew Gervay

Dated

4 September 2000



GB9929835.8

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA AB,
Incorporated in Sweden,
S-151 85 Sodertalje,
Sweden

[ADP No. 07822448003]

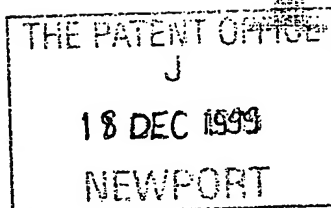
GB9929835.8

By virtue of a direction given under Section of the Patents Act 1977, the application is proceeding in the name of

ASTRAZENECA UK LIMITED
Incorporated in the United Kingdom
15 Stanhope Gate
LONDON
W1Y 6PA
United Kingdom

[ADP No. 07810294001]

The Patent Office



The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference PHM 99-177 9929835.8

2. Patent application number
(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Zeneca Limited
15 Stanhope Gate
LONDON W1Y 6LN
Great Britain

Patents ADP number (if you know it)

If the applicant is a corporate body, give the country/state of its incorporation

6661 330 81
9/3/00
SECTION 30 (1977 ACT) APPLICATION FILED
6254007002

4. Title of the invention CHEMICAL COMPOUNDS

5. Name of your agent (if you have one)

TAIT, Brian Steele

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Global Intellectual Property, Patents
AstraZeneca PLC
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4TG
Great Britain

Patents ADP number (if you know it)

5684600002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country	Priority application number (if you know it)	Date of filing (day / month / year)
---------	---	--

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing (day / month / year)
-------------------------------	--

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or

Do not count copies of the same document

Continuation sheets of this form

Description

34

Claim(s)

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/We request the grant of a patent on the basis of this application.

Signature

Lynda M Slack

Date

17 Dec 99

12. Name and daytime telephone number of person to contact in the United Kingdom

Lynda M Slack

01625 516173

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.

CHEMICAL COMPOUNDS

The present invention relates to compounds which elevate pyruvate dehydrogenase (PDH) activity, processes for their preparation, pharmaceutical compositions containing them
5 as active ingredient, methods for the treatment of disease states associated with reduced PDH activity, to their use as medicaments and to their use in the manufacture of medicaments for use in the elevation of PDH activity in warm-blooded animals such as humans.

Within tissues adenosine triphosphate (ATP) provides the energy for synthesis of complex molecules and, in muscle, for contraction. ATP is generated from the breakdown of
10 energy-rich substrates such as glucose or long chain free fatty acids. In oxidative tissues such as muscle the majority of the ATP is generated from acetyl CoA which enters the citric acid cycle, thus the supply of acetyl CoA is a critical determinant of ATP production in oxidative tissues. Acetyl CoA is produced either by β -oxidation of fatty acids or as a result of glucose metabolism by the glycolytic pathway. The key regulatory enzyme in controlling the rate of
15 acetyl CoA formation from glucose is PDH which catalyses the oxidation of pyruvate to acetyl CoA and carbon dioxide with concomitant reduction of nicotinamide adenine dinucleotide (NAD) to NADH.

In disease states such as both non-insulin dependent (NIDDM) and insulin-dependent diabetes mellitus (IDDM), oxidation of lipids is increased with a concomitant reduction in
20 utilisation of glucose, which contributes to the hyperglycaemia. Reduced glucose utilisation in both IDDM and NIDDM is associated with a reduction in PDH activity. In addition, a further consequence of reduced PDH activity may be that an increase in pyruvate concentration results in increased availability of lactate as a substrate for hepatic gluconeogenesis. It is reasonable to expect that increasing the activity of PDH could increase the rate of glucose
25 oxidation and hence overall glucose utilisation, in addition to reducing hepatic glucose output. Another factor contributing to diabetes mellitus is impaired insulin secretion, which has been shown to be associated with reduced PDH activity in pancreatic β -cells (in a rodent genetic model of diabetes mellitus Zhou et al. (1996) Diabetes 45: 580-586).

Oxidation of glucose is capable of yielding more molecules of ATP per mole of
30 oxygen than is oxidation of fatty acids. In conditions where energy demand may exceed energy supply, such as myocardial ischaemia, intermittent claudication, cerebral ischaemia

and reperfusion, (Zaidan et al., 1998; J. Neurochem. 70: 233-241), shifting the balance of substrate utilisation in favour of glucose metabolism by elevating PDH activity may be expected to improve the ability to maintain ATP levels and hence function.

An agent which is capable of elevating PDH activity may also be expected to be of benefit in treating conditions where an excess of circulating lactic acid is manifest such as in certain cases of sepsis.

The agent dichloroacetic acid (DCA) which increases the activity of PDH after acute administration in animals, (Vary et al., 1988; Circ. Shock, 24: 3-18), has been shown to have the predicted effects in reducing glycaemia, (Stacpoole et al., 1978; N. Engl. J. Med. 298: 526-530), and as a therapy for myocardial ischaemia (Bersin and Stacpoole 1997; American Heart Journal, 134: 841-855) and lactic acidemia, (Stacpoole et al., 1983; N. Engl. J. Med. 309: 390-396).

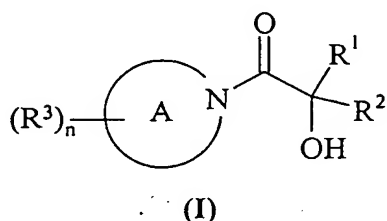
PDH is an intramitochondrial multienzyme complex consisting of multiple copies of several subunits including three enzyme activities E1, E2 and E3, required for the completion of the conversion of pyruvate to acetyl CoA (Patel and Roche 1990; FASEB J., 4: 3224-3233). E1 catalyses the non-reversible removal of CO₂ from pyruvate; E2 forms acetyl CoA and E3 reduces NAD to NADH. Two additional enzyme activities are associated with the complex: a specific kinase which is capable of phosphorylating E1 at three serine residues and a loosely-associated specific phosphatase which reverses the phosphorylation. Phosphorylation of a single one of the three serine residues renders the E1 inactive. The proportion of the PDH in its active (dephosphorylated) state is determined by a balance between the activity of the kinase and phosphatase. The activity of the kinase may be regulated *in vivo* by the relative concentrations of metabolic substrates such as NAD/NADH, CoA/acetylCoA and adenine diphosphate (ADP)/ATP as well as by the availability of pyruvate itself.

European Patent Publication Nos. 617010 and 524781 describes compounds which are capable of relaxing bladder smooth muscle and which may be used in the treatment of urge incontinence. We have found that the compounds of the present invention are very good at elevating PDH activity, a property nowhere disclosed in EP 0617010 and EP 524781.

The present invention is based on the surprising discovery that certain compounds elevate PDH activity, a property of value in the treatment of disease states associated with disorders of glucose utilisation such as diabetes mellitus, obesity, (Curto et al., 1997; Int. J.

Obes. 21: 1137-1142), and lactic acidemia. Additionally the compounds may be expected to have utility in diseases where supply of energy-rich substrates to tissues is limiting such as peripheral vascular disease, (including intermittent claudication), cardiac failure and certain cardiac myopathies, muscle weakness, hyperlipidaemias and atherosclerosis (Stacpoole et al., 1978; N. Engl. J. Med. 298: 526-530). A compound that activates PDH may also be useful in treating Alzheimer's disease (AD) (J Neural Transm (1998) 105, 855-870).

Accordingly, the present invention provides a compound of formula (I):



10 wherein:

Ring A is a nitrogen linked mono or bicyclic heterocyclic ring; wherein if said heterocyclic group contains an -NH- moiety that nitrogen is optionally substituted by R⁴-D-;

R¹ and **R²** are independently C_kalkyl optionally substituted by 1 to 2k+1 atoms selected from fluoro and chloro wherein k is 1-3;

15 or **R¹** and **R²** together with the carbon atom to which they are attached, form a C_mcycloalkyl ring optionally substituted by 1 to 2m-2 fluorine atoms wherein m is 3-5;

R³ is a substituent on carbon and is halo, hydroxy, cyano, formyl, amino, nitro, carboxy, carbamoyl, ureido, thiol, sulphonamoyl or R⁵-E-;

R⁴ is C₁₋₆alkyl, phenyl or a heterocyclic group, wherein in **R⁴** any C₁₋₆alkyl, phenyl or
20 heterocyclic groups (on a ring carbon) may be optionally substituted by one or more R⁶ and if said heterocyclic group contains an -NH- moiety that nitrogen may be optionally substituted by a group selected from R⁸;

D is -C(O)-, -N(R⁹)C(O)-, -S(O)₂-, -NS(O)₂-, -OC(O)- or D is a direct bond;

R⁵ is C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, phenyl, naphthyl or a
25 heterocyclic group, wherein in **R⁵** any C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl, phenyl, naphthyl or heterocyclic groups (on a ring carbon) may be optionally substituted by one or more R⁶ and if said heterocyclic group contains an -NH- moiety that nitrogen may be optionally substituted by a group selected from R⁸;

E is -O-, -N(R⁹)-, -C(O)-, -N(R⁹)C(O)-, -C(O)N(R⁹)-, -S(O)_a- wherein a is 0-2, -OC(O)-, -C(O)O-, -N(R⁹)C(O)O-, -OC(O)N(R⁹)-, -C(S)N(R⁹)-, -N(R⁹)C(S)-, -SO₂N(R⁹)-, -N(R⁹)SO₂-, -N(R⁹)C(O)N(R⁹)-, -N(R⁹)C(S)N(R⁹)-, -SO₂NHC(O)-, -SO₂N(R⁹)C(O)-, -C(O)NHSO₂- or E is a direct bond;

- 5 R⁶ is trifluoromethyl, C₁₋₆alkyl, halo, hydroxy, trifluoromethoxy, cyano, C₁₋₆alkoxy, formyl, C₁₋₆alkanoyl, C₁₋₆alkanoyloxy, amino, *N*-(C₁₋₆alkyl)amino, *N*-(C₁₋₆alkyl)₂amino, C₁₋₆alkanoylamino, C₁₋₆alkanoyl(*N*-C₁₋₆alkyl)amino, nitro, carboxy, carbamoyl, C₁₋₆alkoxycarbonyl, thiol, C₁₋₆alkylsulphanyl, C₁₋₆alkylsulphinyl, C₁₋₆alkylsulphonyl, C₁₋₆alkylsulphonylamino, sulphamoyl, *N*-(C₁₋₆alkyl)aminosulphonyl,
- 10 *N*-(C₁₋₆alkyl)₂aminosulphonyl, *N*-(C₁₋₆alkyl)carbamoyl, *N*-(C₁₋₆alkyl)₂carbamoyl, ureido, *N'*-(C₁₋₆alkyl)ureido or *N'*-(C₁₋₆alkyl)₂ureido, C₂₋₆alkenyl, C₂₋₆alkynyl or C₃₋₆cycloalkyl, naphthyl, phenyl or a heterocyclic group wherein in R⁶ any C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cylcoalkyl, naphthyl, phenyl or heterocyclic groups (on a ring carbon) may be optionally substituted by one or more R⁷ and if said heterocyclic group contains an -NH- moiety that
- 15 nitrogen may be optionally substituted by a group selected from R⁸;

- R⁷ is trifluoromethyl, cyano, C₁₋₆alkyl, halo, hydroxy, trifluoromethoxy, C₁₋₆alkoxy, formyl, C₁₋₆alkanoyl, C₁₋₆alkanoyloxy, amino, *N*-(C₁₋₆alkyl)amino, *N*-(C₁₋₆alkyl)₂amino, C₁₋₆alkanoylamino, C₁₋₆alkanoyl(*N*-C₁₋₆alkyl)amino, nitro, carboxy, carbamoyl, C₁₋₆alkoxycarbonyl, thiol, C₁₋₆alkylsulphanyl, C₁₋₆alkylsulphinyl, C₁₋₆alkylsulphonyl,
- 20 C₁₋₆alkylsulphonylamino, sulphamoyl, *N*-(C₁₋₆alkyl)aminosulphonyl, *N*-(C₁₋₆alkyl)₂aminosulphonyl, *N*-(C₁₋₆alkyl)carbamoyl, *N*-(C₁₋₆alkyl)₂carbamoyl, C₂₋₆alkenyl, C₂₋₆alkynyl, C₃₋₆cycloalkyl or a heterocyclic group (optionally substituted by one or more R¹¹), and wherein in R⁷ any C₁₋₆alkyl, C₂₋₆alkenyl, C₂₋₆alkynyl or C₃₋₆cylcoalkyl groups may be optionally substituted by one or more groups selected from R¹²;
- 25 R⁸ is C₁₋₆alkyl, C₁₋₆alkanoyl, C₁₋₆alkylsulphonyl, C₁₋₆alkoxycarbonyl, carbamoyl, *N*-(C₁₋₆alkyl)carbamoyl, *N,N*-(C₁₋₆alkyl)₂carbamoyl, benzoyl, (heterocyclic group)carbonyl, phenylsulphonyl, (heterocyclic group)sulphonyl, phenyl or a carbon linked heterocyclic group, and wherein in R⁸ any C₁₋₆alkyl, phenyl or heterocyclic group (on a ring carbon) may be optionally substituted by one or more R⁶, and if a heterocyclic group contains an -NH-
- 30 moiety that nitrogen may be optionally substituted by a group selected from R¹¹;

R^9 is hydrogen or C_{1-6} alkyl optionally substituted by one or more R^{10} with the proviso that R^{10} is not a substituent on the carbon attached to a nitrogen atom;

R^{10} is halo, hydroxy, amino, cyano, nitro, trifluoromethyl, trifluoromethoxy, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, N -(C_{1-6} alkyl)amino, N -(C_{1-6} alkyl)₂amino, C_{1-6} alkanoylamino,
 5 C_{1-6} alkanoyl(N - C_{1-6} alkyl)amino, C_{1-6} alkylsulphonylamino, C_{1-6} alkylsulphonyl(N - C_{1-6} alkyl)amino, thiol, C_{1-6} alkylsulphanyl, C_{1-6} alkylsulphinyl, C_{1-6} alkylsulphonyl, sulphamoyl, N -(C_{1-6} alkyl)aminosulphonyl, N -(C_{1-6} alkyl)₂aminosulphonyl, carboxy, carbamoyl, N -(C_{1-6} alkyl)carbamoyl, N -(C_{1-6} alkyl)₂carbamoyl, C_{1-6} alkoxycarbonyl, C_{1-6} alkanoyl or formyl;

10 R^{11} is C_{1-6} alkyl, C_{1-6} alkanoyl, C_{1-6} alkylsulphonyl, C_{1-6} alkoxycarbonyl, carbamoyl, N -(C_{1-6} alkyl)carbamoyl, N,N -(C_{1-6} alkyl)₂carbamoyl, C_{1-6} alkoxy C_{1-6} alkanoyl, phenyl C_{1-6} alkyl, benzoyl, phenyl C_{1-6} alkanoyl, phenyl C_{1-6} alkoxycarbonyl or phenylsulphonyl and wherein in R^{11} any C_{1-6} alkyl group can be optionally substituted by one or more R^{13} ;

R^{12} is halo, hydroxy, N -methylpiperazinyl, N -acetylpiperazinyl, morpholino,
 15 piperidino, cyano, amino, N,N -dimethylamino, acetamido, carbamoyl, carboxy, methanesulphonyl or sulphamoyl;

R^{13} is halo, hydroxy, cyano, amino, N,N -dimethylamino, acetamido, carbamoyl, carboxy, methanesulphonyl or sulphamoyl;

n is 0-5; wherein the values of R^3 may be the same or different;

20 or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof; with the proviso that if R^1 is methyl, R^2 is trifluoromethyl and Ring A is piperazin-1-yl then $(R^3)_n$ is not i) 4-cyanobenzoyl, ii) 2-methyl-4-benzoyloxycarbonyl, iii) 2-methyl, iv) 2-methyl-4-cyanobenzoyl, v) 2,5-dimethyl-4-benzyl, vi) 2,5-dimethyl or vii) 2,5-dimethyl-4-cyanobenzoyl.

25 In this specification the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. For example, " C_{1-6} alkyl" includes C_{1-4} alkyl, methyl, ethyl, propyl, isopropyl and *t*-butyl. However, references to individual alkyl groups such as 'propyl' are specific for the straight chained version only and references to individual branched chain alkyl
 30 groups such as 'isopropyl' are specific for the branched chain version only. The term "halo" refers to fluoro, chloro, bromo and iodo. Where a phrase such as "any C_{1-6} alkyl group may be

optionally substituted by one or more groups" for the avoidance of doubt, it is to be understood that this refers to all groups that contain a C₁₋₆alkyl group, for example this phrase would also relate to a C₁₋₆alkanoyl group if that was listed in the paragraph.

A "nitrogen linked mono or bicyclic heterocyclic ring" is a saturated, partially
 5 saturated or fully unsaturated, mono or bicyclic ring containing 4-12 atoms, one atom of which is a nitrogen atom (attached to form an amide as shown) and the other atoms are either all carbon atoms or they are carbon atoms and 1-3 heteroatoms chosen from nitrogen, sulphur or oxygen, wherein a -CH₂- group can optionally be replaced by a -C(O)- and a ring nitrogen and/or sulphur atom may be optionally oxidised to form the *N*-oxide and or the S-oxides. It
 10 will be appreciated that in forming this nitrogen link, the nitrogen atom is not quaternised, i.e. a neutral compound is formed.

A "heterocyclic group" is a saturated, partially saturated or unsaturated, mono or bicyclic ring containing 4-12 atoms of which at least one atom is chosen from nitrogen, sulphur or oxygen, which may, unless otherwise specified, be carbon or nitrogen linked,
 15 wherein a -CH₂- group can optionally be replaced by a -C(O)- and a ring nitrogen and/or sulphur atom may be optionally oxidised to form the *N*-oxide and or the S-oxides. Suitable values for a "heterocyclic group" include morpholino, piperidyl, pyridyl, pyranyl, pyrrolyl, isothiazolyl, indolyl, quinolyl, thienyl, 1,3-benzodioxolyl, thiadiazolyl, piperazinyl, thiazolidinyl, pyrrolidinyl, thiomorpholino, pyrrolinyl, homopiperazinyl, tetrahydropyranyl,
 20 imidazolyl, pyrimidyl, pyrazinyl, pyridazinyl, isoxazolyl, 4-pyridone, 1-isoquinolone, 2-pyrrolidone, 4-thiazolidone, pyridine-*N*-oxide and quinoline-*N*-oxide.

An example of "C₁₋₆alkanoyloxy" is acetoxy. Examples of "C₁₋₆alkoxycarbonyl" include methoxycarbonyl, ethoxycarbonyl, *n*- and *t*-butoxycarbonyl. Examples of "C₁₋₆alkoxy" include methoxy, ethoxy and propoxy. Examples of "C₁₋₆alkanoylamino" include
 25 formamido, acetamido and propionylamino. Examples of "C₁₋₆alkylsulphanyl" include methylthio and ethylthio. Examples of "C₁₋₆alkylsulphinyl" include methylsulphinyl and ethylsulphinyl. Examples of "C₁₋₆alkylsulphonyl" include C₁₋₄alkylsulphonyl, mesyl and ethylsulphonyl. Examples of "C₁₋₆alkylsulphonylamino" include C₁₋₄alkylsulphonylamino, mesylamino and ethylsulphonylamino. Examples of "C₁₋₆alkanoyl" include C₁₋₄alkanoyl,
 30 propionyl and acetyl. Examples of "*N*-(C₁₋₆alkyl)amino" include methylamino and ethylamino. Examples of "*N*-(C₁₋₆alkyl)₂amino" include di-*N*-methylamino, di-(*N*-ethyl)amino

- “C₂₋₆alkynyl” are C₂₋₄alkynyl, ethynyl, 1-propynyl and 2-propynyl. Examples of “C₁₋₆alkanoyl(*N*-C₁₋₆alkyl)amino” are C₁₋₄alkanoyl(*N*-C₁₋₄alkyl)amino, (*N*-methyl)formamido and (*N*-propyl)acetamido. Examples of “*N*-(C₁₋₆alkyl)aminosulphonyl” are *N*-(C₁₋₄alkyl)aminosulphonyl, *N*-(methyl)aminosulphonyl and *N*-(ethyl)aminosulphonyl.
- 5 Examples of “*N*-(C₁₋₆alkyl)₂aminosulphonyl” are *N*-(C₁₋₄alkyl)₂aminosulphonyl, *N*-(dimethyl)aminosulphonyl and *N*-(methyl)-*N*-(ethyl)aminosulphonyl. Examples of “*N*-(C₁₋₆alkyl)carbamoyl” are *N*-(C₁₋₄alkyl)carbamoyl, methylaminocarbonyl and ethylaminocarbonyl. Examples of “*N*-(C₁₋₆alkyl)₂carbamoyl” are *N*-(C₁₋₄alkyl)₂carbamoyl, dimethylaminocarbonyl and *N*-methyl-*N*-ethylaminocarbonyl. Examples of “(heterocyclic
- 10 group)carbonyl” are pyrid-3-ylcarbonyl and pyrimid-2-ylcarbonyl. Examples of “(heterocyclic group)sulphonyl” are pyrid-3-ylsulphonyl and pyrimid-2-ylsulphonyl. Examples of “C₁₋₆alkoxyC₁₋₆alkanoyl” include ethoxypropionyl and methoxyacetyl. Examples of “phenylC₁₋₆alkyl” include phenethyl and benzyl. Examples of “phenylC₁₋₆alkanoyl” include 3-phenylpropionyl and phenacetyl. Examples of “phenylC₁₋₆alkoxycarbonyl” include
- 15 benzyloxyoxycarbonyl and 2-phenylethoxycarbonyl. Examples of “*N*’-(C₁₋₆alkyl)ureido” are *N*’-methylureido and *N*’-ethylureido. Examples of “*N*’-(C₁₋₆alkyl)₂ureido” are *N*’,*N*’-dimethylureido and *N*’-methyl-*N*’-ethylureido.

Preferred values of Ring A, R¹, R², R³ and n are as follows. Such values may be used where appropriate with any of the definitions, claims or embodiments defined hereinbefore or

20 hereinafter.

Particularly R⁴ is ethyl, 4-mesylphenyl, 4-methylthiophenyl, 4-carboxyphenyl, 4-(*N,N*-dimethylcarbamoyl)phenyl, 4-fluorophenyl, 4-(2-hydroxyethylamino)phenyl, 4-cyanophenyl, 2-chloro-4-cyanophenyl, 4-acetylphenyl, 4-methoxyphenyl, 4-(*N,N*-dimethylamino)phenyl, 4-bromophenyl or thien-2-yl.

25 Particularly D is -SO₂- or -C(O)-.

In a further aspect of the invention more preferably R⁴-D- is ethylsulphonyl, 4-carboxyphenylsulphonyl, 4-(*N,N*-dimethylcarbamoyl)phenylsulphonyl, 4-fluorophenylsulphonyl, 4-fluorobenzoyl, thien-2-ylsulphonyl, 4-(*N,N*-dimethylcarbamoyl)benzoyl, 4-(2-hydroxyethylamino)phenylsulphonyl,

30 4-cyanophenylsulphonyl, 2-chloro-4-cyanophenylsulphonyl, 4-acetylphenylsulphonyl,

4-methoxyphenylsulphonyl, 4-methoxybenzoyl, 4-(*N,N*-dimethylamino)benzoyl, 4-bromophenylsulphonyl or 4-bromobenzoyl.

In a further aspect of the invention R^5 is 4-mesylphenyl, 4-methylthiophenyl, 4-fluorophenyl or 4-cyanophenyl.

5 In another aspect of the invention E is $-SO_2-$, $-S-$, $-C(O)-$ or $-C(O)NH-$.

A preferred compound of the invention is any one of the Examples or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof.

Preferred aspects of the invention are those which relate to the compound or a pharmaceutically acceptable salt thereof.

10 Within the present invention it is to be understood that a compound of the formula (I) or a salt thereof may exhibit the phenomenon of tautomerism and that the formulae drawings within this specification can represent only one of the possible tautomeric forms. It is to be understood that the invention encompasses any tautomeric form which elevates PDH activity and is not to be limited merely to any one tautomeric form utilized within the formulae
15 drawings. The formulae drawings within this specification can represent only one of the possible tautomeric forms and it is to be understood that the specification encompasses all possible tautomeric forms of the compounds drawn not just those forms which it has been possible to show graphically herein.

It will be appreciated by those skilled in the art that certain compounds of formula (I)
20 contain one or more asymmetrically substituted carbon and/or sulphur atoms, and accordingly may exist in, and be isolated as enantiomerically pure, a mixture of diastereoisomers or as a racemate. It will also be appreciated by those skilled in the art that certain compounds of formula (I) contain substituents on a saturated heterocycle that may bear a *syn*- or *anti*- (or *cis*- and *trans*) relationship. It is to be understood that the present invention encompasses all
25 such geometric isomers. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, enantiomerically pure, mixture of diastereoisomers, polymorphic or stereoisomeric form, or mixtures thereof, which form possesses properties useful in the elevation of PDH activity, it being well known in the art how to prepare optically-active forms (for example, by resolution of the racemic form by
30 recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, by enzymatic resolution, (for example WO 9738124), by biotransformation, or by

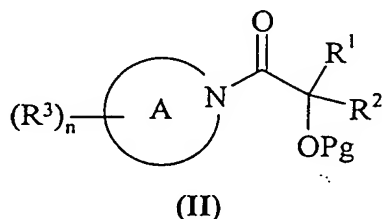
chromatographic separation using a chiral stationary phase) and how to determine efficacy for the elevation of PDH activity by the standard tests described hereinafter.

It is also to be understood that certain compounds of the formula (I) and salts thereof can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms which elevate PDH activity.

A compound of the formula (I), or salt thereof, and other compounds of the invention (as hereinafter defined) may be prepared by any process known to be applicable to the preparation of chemically-related compounds. Such processes include, for example, those illustrated in European Patent Applications, Publication Nos. 0524781, 0617010, 0625516, and in GB 2278054, WO 9323358 and WO 9738124.

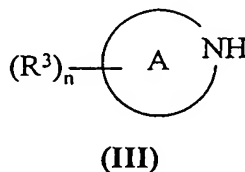
Another aspect of the present invention provides a process for preparing a compound of formula (I) or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof, which process (in which variable groups are as defined for formula (I) unless otherwise stated) comprises of:

(a) deprotecting a protected compound of formula (II):

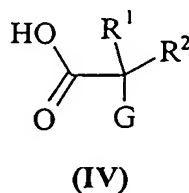


where Pg is an alcohol protecting group;

(b) by coupling an amine of formula (III):



with an acid of formula (IV):



wherein G is a hydroxyl group;

(c) by coupling an amine of formula (III) with an activated acid derivative of formula (IV)

wherein G is a hydroxyl group which may be protected as an ester or ether;

and thereafter if necessary:

- 5 i) converting a compound of the formula (I) into another compound of the formula (I);
- ii) removing any protecting groups; or
- iii) forming a pharmaceutically acceptable salt or *in vivo* hydrolysable ester.

Suitable values for Pg are a benzyl group, a silyl group or an acetyl protecting group.

Specific conditions of the above reactions are as follows:

10 Process a)

Suitable reagents for deprotecting an alcohol of formula (II) are for example:

1) when Pg is benzyl:

- (i) hydrogen in the presence of palladium/carbon catalyst, i.e. hydrogenolysis; or
- (ii) hydrogen bromide or hydrogen iodide;

15 2) when Pg is a silyl protecting group:

- (i) tetrabutylammonium fluoride; or
- (ii) aqueous hydrofluoric acid;

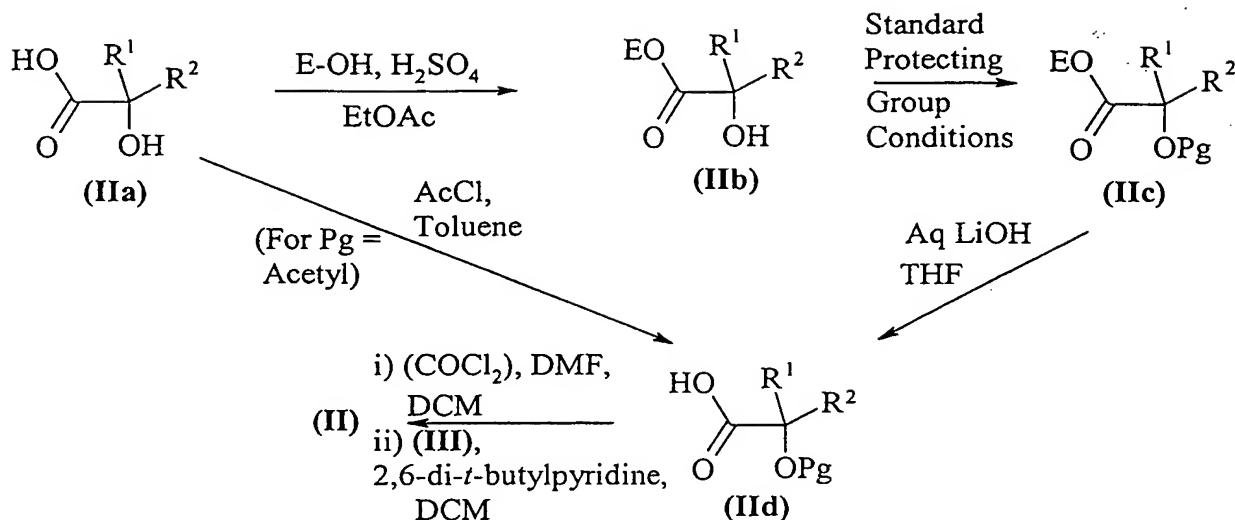
3) when Pg is acetyl:

- i) mild aqueous base for example lithium hydroxide; or

20 ii) ammonia or an amine such as dimethylamine.

The reaction can be conducted in a suitable solvent such as ethanol, methanol, acetonitrile, or dimethylsulphoxide and may conveniently be performed at a temperature in the range of -40 to 100°C.

Compounds of formula (II) may be prepared according to the following scheme:



Scheme 1

E is a carboxy protecting group. Suitable values for E include C_{1-6} alkyl, such as methyl and ethyl.

- 5 Compounds of formula (IIa) and (III) are commercially available compounds, or they are known in the literature, or they are prepared by standard processes known in the art.

Process b)

- An amine of formula (III) and an acid of formula (IV) may be coupled together in the presence of a suitable coupling reagent. Standard peptide coupling reagents known in the art
- 10 can be employed as suitable coupling reagents, for example conditions such as those described above for the coupling of (IIc) and (III), or carbonyldiimidazole and dicyclohexyl-carbodiimide, optionally in the presence of a catalyst such as dimethylaminopyridine or 4-pyrrolidinopyridine, optionally in the presence of a base for example triethylamine, pyridine, or 2,6-di-*alkyl*-pyridines such as 2,6-lutidine or
- 15 2,6-di-*tert*-butylpyridine. Suitable solvents include dimethylacetamide, dichloromethane, benzene, tetrahydrofuran, and dimethylformamide. The coupling reaction may conveniently be performed at a temperature in the range of -40 to 40°C.

Compounds of formula (IV) are commercially available compounds, or they are known in the literature, or they are prepared by standard processes known in the art.

- 20 If the resolved acid of formula (IV) is required it may be prepared by any of the known methods for preparation of optically-active forms (for example, by recrystallization of the chiral salt {for example WO 9738124}, by enzymatic resolution, by biotransformation, or

by chromatographic separation using a chiral stationary phase). For example if an (R)-(+)
resolved acid is required it may be prepared by the method of Scheme 2 in World Patent
Application Publication No. WO 9738124 for preparation of the (S)-(-) acid, i.e. using the
classical resolution method described in European Patent Application Publication No. EP
5 0524781, also for preparation of the (S)-(-) acid, except that (1S,2R)-norephedrine may be
used in place of (S)-(-)-1-phenylethylamine. The chiral acid may also be prepared by using the
enzymatic resolution method as described in Tetrahedron Asymmetry, 1999, 10, 679.

Process c)

An amine of formula (III) may be coupled with an activated acid derivative of formula
10 (IV) for example acid chlorides, acid anhydrides, or phenyl esters, wherein G is a hydroxyl
group which may be suitably protected as a stable ester or ether. This coupling may be
achieved optionally in the presence of a base for example triethylamine, pyridine, or
2,6-di-*alkyl*-pyridines such as 2,6-lutidine or 2,6-di-*tert*-butylpyridine. Suitable solvents
include dimethylacetamide, dichloromethane, benzene, tetrahydrofuran, and
15 dimethylformamide. The coupling reaction may conveniently be performed at a temperature
in the range of -40 to 40°C;

If not commercially available, the necessary starting materials for the procedures such
as that described above may be made by procedures which are selected from standard organic
chemical techniques, techniques which are analogous to the synthesis of known, structurally
20 similar compounds, or techniques which are analogous to the above described procedure or
the procedures described in the examples.

For example, it will be appreciated that certain of the optional aromatic substituents in
the compounds of the present invention may be introduced by standard aromatic substitution
reactions or generated by conventional functional group modifications or interconversions
25 either prior to or immediately following the processes mentioned above, and as such are
included in the process aspect of the invention. Such reactions and modifications include, for
example, introduction of a substituent by means of an aromatic substitution reaction,
reduction of substituents, alkylation of substituents and oxidation of substituents. The reagents
and reaction conditions for such procedures are well known in the chemical art. Particular
30 examples of aromatic substitution reactions include the introduction of a nitro group using
concentrated nitric acid, the introduction of an acyl group using, for example, an acylhalide

and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; the introduction of an alkyl group using an alkyl halide and Lewis acid (such as aluminium trichloride) under Friedel Crafts conditions; and the introduction of a halogeno group.

Particular examples of modifications include the reduction of a nitro group to an amino group
5 by, for example, catalytic hydrogenation with a nickel catalyst or treatment with iron in the presence of hydrochloric acid with heating; oxidation of alkylthio to alkylsulphinyl or alkylsulphonyl using, for example, hydrogen peroxide in acetic acid with heating or 3-chloroperbenzoic acid. Particular examples of functional group interconversions are for example conversion of an aniline into a halophenyl by, for example, diazotisation in the
10 presence of cuprous halides.

It is noted that many of the starting materials for synthetic methods as described above are commercially available and/or widely reported in the scientific literature, or could be made from commercially available compounds using adaptations of processes reported in the scientific literature.

15 It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

20 A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example benzoyl. The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting
25 group. Thus, for example, an acyl group such as an alkanoyl or alkoxycarbonyl group or an aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid such as, for example hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid and an
30 arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation in the presence of a catalyst such as palladium-on-carbon, or by

treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group for a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

- 5 A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with
10 a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation in the presence of a catalyst such as palladium-on-carbon.

- A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis
15 with a base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

- The protecting groups may be removed at any convenient stage in the synthesis using
20 conventional techniques well known in the chemical art.

- In cases where compounds of formula (I) are sufficiently basic or acidic to form stable acid or basic salts, administration of the compound as a salt may be appropriate, and pharmaceutically acceptable salts may be made by conventional methods such as those described following. Examples of suitable pharmaceutically acceptable salts are organic acid
25 addition salts formed with acids which form a physiologically acceptable anion, for example, tosylate, methanesulphonate, acetate, tartrate, citrate, succinate, benzoate, ascorbate, α -ketoglutarate, and α -glycerophosphate. Suitable inorganic salts may also be formed such as sulphate, nitrate, and hydrochloride.

- Pharmaceutically acceptable salts may be obtained using standard procedures well
30 known in the art, for example by reacting a sufficiently basic compound of formula (I) (or its ester) with a suitable acid affording a physiologically acceptable anion. It is also possible with

most compounds of the invention to make a corresponding alkali metal (e.g. sodium, potassium, or lithium) or alkaline earth metal (e.g. calcium) salt by treating a compound of formula (I) (and in some cases the ester) with one equivalent of an alkali metal or alkaline earth metal hydroxide or alkoxide (e.g. the ethoxide or methoxide) in aqueous medium

5 followed by conventional purification techniques.

The compounds of the formula (I) may be administered in the form of a prodrug which is broken down in the human or animal body to give a compound of the formula (I). Examples of prodrugs include *in vivo* hydrolysable esters of a compound of the formula (I).

10 An *in vivo* hydrolysable ester of a compound of the formula (I) containing carboxy or hydroxy group is, for example, a pharmaceutically acceptable ester which is hydrolysed in the human or animal body to produce the parent acid or alcohol.

Suitable *in vivo* hydrolysable esters for a compound of the formula (I) containing a carboxy group include C₁₋₆alkoxymethyl esters for example methoxymethyl, C₁₋₆alkanoyloxymethyl esters for example pivaloyloxymethyl, phthalidyl esters, 15 C₃₋₈cycloalkoxycarbonyloxyC₁₋₆alkyl esters for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolen-2-onylmethyl esters for example 5-methyl-1,3-dioxolen-2-onylmethyl; and C₁₋₆alkoxycarbonyloxyethyl esters for example 1-methoxycarbonyloxyethyl and may be formed at any carboxy group in the compounds of this invention.

Suitable *in vivo* hydrolysable esters of a compound of the formula (I) containing a 20 hydroxy group includes inorganic esters such as phosphate esters and α -acyloxyalkyl ethers. Examples of α -acyloxyalkyl ethers include acetoxymethoxy and 2,2-dimethylpropionyloxymethoxy. Other *in vivo* hydrolysable ester forming groups for hydroxy include alkanoyl, benzoyl, phenylacetyl and substituted benzoyl and phenylacetyl, alkoxy carbonyl (to give alkyl carbonate esters), dialkylcarbamoyl and 25 *N*-(dialkylaminoethyl)-*N*-alkylcarbamoyl (to give carbamates), dialkylaminoacetyl and carboxyacetyl. Examples of substituents for benzoyl include morpholino and piperazino linked from a ring nitrogen atom via a methylene group to the 3- or 4- position of the benzoyl ring.

In vivo cleavable prodrugs of compounds of formula (I) also include *in vivo* 30 hydrolysable amides of compounds of the formula (I) containing a carboxy group, for example, a *N*-C₁₋₆alkyl or *N*-di-C₁₋₆alkyl amide such as *N*-methyl, *N*-ethyl, *N*-propyl,

N-dimethyl, *N*-ethyl-*N*-methyl or *N*-diethyl amide.

The identification of compounds which elevate PDH activity is the subject of the present invention. These properties may be assessed, for example, using one or more of the procedures set out below:

5 (a) In vitro elevation of PDH activity

This assay determines the ability of a test compound to elevate PDH activity. cDNA encoding PDH kinase may be obtained by Polymerase Chain Reaction (PCR) and subsequent cloning. This may be expressed in a suitable expression system to obtain polypeptide with PDH kinase activity. For example rat PDHkinaseII (rPDHKII) obtained by expression of
10 recombinant protein in *Escherichia coli* (*E. Coli*), was found to display PDH kinase activity.

In the case of the rPDHKII (Genbank accession number U10357) a 1.3kb fragment encoding the protein was isolated by PCR from rat liver cDNA and cloned into a vector (for example pQE32 - Quiagen Ltd.). The recombinant construct was transformed into *E. coli* (for example M15pRep4 - Quiagen Ltd.). Recombinant clones were identified, plasmid DNA was
15 isolated and subjected to DNA sequence analysis. One clone which had the expected nucleic acid sequence was selected for the expression work. Details of the methods for the assembly of recombinant DNA molecules and the expression of recombinant proteins in bacterial systems can be found in standard texts for example Sambrook et al, 1989, Molecular Cloning - A Laboratory Manual, 2nd edition, Cold Spring Harbour Laboratory Press. Other known
20 PDH kinases for use in assays, may be cloned and expressed in a similar manner.

For expression of rPDHKII activity, *E. coli* strain M15pRep4 cells were transformed with the pQE32 vector containing rPDHKII cDNA. This vector incorporates a 6-His tag onto the protein at its N-terminus. *E. coli* were grown to an optical density of 0.6 (600 nm) and protein expression was induced by the addition of 10 μ M isopropylthio- β -galactosidase. Cells
25 were grown for 18 hours at 18°C and harvested by centrifugation. The resuspended cell paste was lysed by homogenisation and insoluble material removed by centrifugation at 24000xg for 1 hour. The 6-His tagged protein was removed from the supernatant using a nickel chelating nitrilotriacetic acid resin (Ni-NTA: Quiagen Ltd.) matrix (Quiagen) which was washed with 20 mM tris(hydroxymethyl)aminomethane-hydrogen chloride, 20 mM
30 imidazole, 0.5 M sodium chloride pH 8.0, prior to elution of bound protein using a buffer containing 20 mM tris(hydroxymethyl)aminomethane-hydrogen chloride, 200 mM imidazole,

0.15 M sodium chloride pH 8.0. Eluted fractions containing 6-His protein were pooled and stored in aliquots at -80°C in 10% glycerol.

Each new batch of stock enzyme was titrated in the assay to determine a concentration giving approximately 90% inhibition of PDH in the conditions of the assay. For a typical

5 batch, stock enzyme was diluted to 7.5µg/ml.

For assay of the activity of novel compounds, compounds were diluted with 10% dimethylsulphoxide (DMSO) and 10µl transferred to individual wells of 96-well assay plates. Control wells contained 20µl 10% DMSO instead of compound. 40µl Buffer containing 50mM potassium phosphate buffer pH 7.0, 10mM ethylene glycol-bis(β-aminoethyl
10 ether)-N,N-tetracetic acid (EGTA), 1mM benzamidine, 1mM phenylmethylsulphonyl fluoride (PMSF), 0.3mM tosyl-L-lysine chloromethyl ketone (TLCK), 2mM dithiothreitol (DTT), recombinant rPDHKII and compounds were incubated in the presence of PDH kinase at room temperature for 45 minutes. In order to determine the maximum rate of the PDH reaction a second series of control wells were included containing 10% DMSO instead of compound and
15 omitting rPDHKII. PDH kinase activity was then initiated by the addition of 5 µM ATP, 2 mM magnesium chloride and 0.04 U/ml PDH (porcine heart PDH Sigma P7032) in a total volume of 50 µl and plates incubated at ambient temperature for a further 45 minutes. The residual activity of the PDH was then determined by the addition of substrates (2.5mM coenzyme A, 2.5mM thiamine pyrophosphate (cocarboxylase), 2.5mM sodium pyruvate,
20 6mM NAD in a total volume of 80µl and the plates incubated for 90 minutes at ambient temperature. The production of reduced NAD (NADH) was established by measured optical density at 340nm using a plate reading spectrophotometer. The ED₅₀ for a test compound was determined in the usual way using results from 12 concentrations of the compound.

(b) In vitro elevation of PDH activity in isolated primary cells

25 This assay determines the ability of compounds to stimulate pyruvate oxidation in primary rat hepatocytes.

Hepatocytes were isolated by the two-step collagenase digestion procedure described by Seglen (Methods Cell Biol. (1976) 13, 29-33) and plated out in 6-well culture plates (Falcon Primaria) at 600000 viable cells per well in Dulbecco's Modified Eagles Medium
30 (DMEM, Gibco BRL) containing 10% foetal calf serum (FCS), 10% penicillin/streptomycin (Gibco BRL) and 10% non-essential amino acids (NEAA, Gibco BRL). After 4 hours

incubation at 37°C in 5% CO₂, the medium was replaced with Minimum Essential Medium (MEM, Gibco BRL) containing NEAA and penicillin/streptomycin as above in addition to 10nM dexamethasone and 10nM insulin.

The following day cells were washed with phosphate buffered saline (PBS) and medium replaced with 1ml HEPES-buffered Krebs solution (25mM HEPES, 0.15M sodium chloride, 25 mM sodium hydrogen carbonate, 5mM potassium chloride, 2mM calcium chloride, 1mM magnesium sulphate, 1 mM potassium dihydrogen phosphate) containing the compound to be tested at the required concentration in 0.1% DMSO. Control wells contained 0.1% DMSO only and a maximum response was determined using a 10 µM treatment of a known active compound. After a preincubation period of 40 minutes at 37°C in 5% CO₂, cells were pulsed with sodium pyruvate to a final concentration of 0.5mM (containing 1-¹⁴C sodium pyruvate (Amersham product CFA85) 0.18Ci/mmol) for 12 minutes. The medium was then removed and transferred to a tube which was immediately sealed with a bung containing a suspended centre well. Absorbent within the centre well was saturated with 50% phenylethylamine, and CO₂ in the medium released by the addition of 0.2µl 60% (w/v) perchloric acid (PCA). Released ¹⁴CO₂ trapped in the absorbent was determined by liquid scintillation counting. The ED₅₀ for a test compound was determined in the usual way using results from 7 concentrations of the compound.

(c) In vivo elevation of PDH activity

The capacity of compounds to increase the activity of PDH in relevant tissues of rats may be measured using the test described hereinafter. Typically an increase in the proportion of PDH in its active, nonphosphorylated form may be detected in muscle, heart, liver and adipose tissue after a single administration of an active compound. This may be expected to lead to a decrease in blood glucose after repeated administration of the compound. For example a single administration of DCA, a compound known to activate PDH by inhibition of PDH kinase (Whitehouse, Cooper and Randle (1974) Biochem. J. 141, 761-774) 150 mg/kg, intraperitoneally, increased the proportion of PDH in its active form (Vary et al. (1988) Circ. Shock 24, 3-18) and after repeated administration resulted in a significant decrease in plasma glucose (Evans and Stacpoole (1982) Biochem. Pharmacol. 31, 1295-1300).

Groups of rats (weight range 140-180g) are treated with a single dose or multiple doses of the compound of interest by oral gavage in an appropriate vehicle. A control group of

rats is treated with vehicle only. At a fixed time after the final administration of compound, animals are terminally anaesthetised, tissues are removed and frozen in liquid nitrogen. For determination of PDH activity, muscle samples are disrupted under liquid nitrogen prior to homogenisation by one thirty-second burst in a Polytron homogenizer in 4 volumes of a
5 buffer containing 40 mM potassium phosphate pH 7.0, 5 mM EDTA, 2mM DTT, 1% Triton X-100, 10mM sodium pyruvate, 10µM phenylmethylsulphonyl chloride (PMSF) and 2µg/ml each of leupeptin, pepstain A and aprotinin. Extracts are centrifuged before assay. A portion of the extract is treated with PDH phosphatase prepared from pig hearts by the method of Siess and Wieland (Eur. J. Biochem (1972) 26, 96): 20 µl extract, 40 µl phosphatase (1:20
10 dilution), in a final volume of 125 µl containing 25 mM magnesium chloride, 1 mM calcium chloride. The activity of the untreated sample is compared with the activity of the dephosphorylated extract thus prepared. PDH activity is assayed by the method of Stansbie et al., (Biochem. J. (1976) 154, 225). 50 µl Extract is incubated with 0.75 mM NAD, 0.2 mM CoA, 1.5 mM thiamine pyrophosphate (TPP) and 1.5mM sodium pyruvate in the presence of
15 20 µg/ml p-(p-amino-phenylazo) benzene sulphonic acid (AABS) and 50 mU/ml arylamine transferase (AAT) in a buffer containing 100 mM tris(hydroxymethyl)aminomethane, 0.5 mM EDTA, 50mM sodium fluoride, 5mM 2-mercaptoethanol and 1mM magnesium chloride pH 7.8. AAT is prepared from pigeon livers by the method of Tabor et al. (J. Biol. Chem. (1953) 204, 127). The rate of acetyl CoA formation is determined by the rate of reduction of AABS
20 which is indicated by a decrease in optical density at 460 nm.

Liver samples are prepared by an essentially similar method, except that sodium pyruvate is excluded from the extraction buffer and added to the phosphatase incubation to a final concentration of 5mM.

Treatment of an animal with an active compound results in an increase in the activity
25 of PDH complex in tissues. This is indicated by an increase in the amount of active PDH (determined by the activity of untreated extract as a percentage of the total PDH activity in the same extract after treatment with phosphatase).

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula (I) as defined hereinbefore or a
30 pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof, in association with a pharmaceutically acceptable excipient or carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) for example as a sterile solution, suspension or emulsion, for topical administration for example as an ointment or cream or for rectal administration for example as
5 a suppository. In general the above compositions may be prepared in a conventional manner using conventional excipients.

The compositions of the present invention are advantageously presented in unit dosage form. The compound will normally be administered to a warm-blooded animal at a unit dose within the range 5-5000 mg per square metre body area of the animal, i.e. approximately
10 0.1-100 mg/kg. A unit dose in the range, for example, 1-100 mg/kg, preferably 1-50 mg/kg is envisaged and this normally provides a therapeutically-effective dose. A unit dose form such as a tablet or capsule will usually contain, for example 1-250 mg of active ingredient.

According to a further aspect of the present invention there is provided a compound of the formula (I) or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof as
15 defined hereinbefore for use in a method of treatment of the human or animal body by therapy.

We have found that compounds of the present invention elevate PDH activity and are therefore of interest for their blood glucose-lowering effects.

A further feature of the present invention is a compound of formula (I) and pharmaceutically acceptable salts or *in vivo* hydrolysable esters thereof for use as a
20 medicament.

Conveniently this is a compound of formula (I), or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof, for use as a medicament for producing an elevation of PDH activity in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a
25 compound of the formula (I), or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof in the manufacture of a medicament for use in the production of an elevation of PDH activity in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method for producing an elevation of PDH activity in a warm-blooded animal, such as a human being, in need of such
30 treatment which comprises administering to said animal an effective amount of a compound of

formula (I) or a pharmaceutically acceptable salt or an *in vivo* hydrolysable ester thereof as defined hereinbefore.

As stated above the size of the dose required for the therapeutic or prophylactic treatment of a particular disease state will necessarily be varied depending on the host treated, the route of administration and the severity of the illness being treated. Preferably a daily dose in the range of 1-50 mg/kg is employed. However the daily dose will necessarily be varied depending upon the host treated, the particular route of administration, and the severity of the illness being treated. Accordingly the optimum dosage may be determined by the practitioner who is treating any particular patient.

- 10 The elevation of PDH activity described herein may be applied as a sole therapy or may involve, in addition to the subject of the present invention, one or more other substances and/or treatments. Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate administration of the individual components of the treatment. For example in the treatment of diabetes mellitus chemotherapy may include the following main
- 15 categories of treatment:
- i) insulin;
 - ii) insulin secretagogue agents designed to stimulate insulin secretion (for example glibenclamide, tolbutamide, other sulphonylureas);
 - iii) oral hypoglycaemic agents such as metformin, thiazolidinediones;
 - 20 iv) agents designed to reduce the absorption of glucose from the intestine (for example acarbose);
 - v) agents designed to treat complications of prolonged hyperglycaemia;
 - vi) other agents used to treat lactic acidemia;
 - vii) inhibitors of fatty acid oxidation;
 - 25 viii) lipid lowering agents;
 - ix) agents used to treat coronary heart disease and peripheral vascular disease such as aspirin, pentoxifylline, cilostazol; and/or
 - x) thiamine.

As stated above the compounds defined in the present invention are of interest for their ability to elevate the activity of PDH. Such compounds of the invention may therefore be useful in a range of disease states including diabetes mellitus, peripheral vascular disease, (including

intermittent claudication), cardiac failure and certain cardiac myopathies, myocardial ischaemia, cerebral ischaemia and reperfusion, muscle weakness, hyperlipidaemias, Alzheimers disease and/or atherosclerosis.

In addition to their use in therapeutic medicine, the compounds of formula (I) and
5 their pharmaceutically acceptable salts are also useful as pharmacological tools in the development and standardisation of *in vitro* and *in vivo* test systems for the evaluation of the effects of elevators of PDH activity in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the search for new therapeutic agents.

The invention will now be illustrated by the following non-limiting examples in
10 which, unless stated otherwise:

- (i) temperatures are given in degrees Celsius (°C); operations were carried out at room or ambient temperature, that is, at a temperature in the range of 18-25°C;
- (ii) organic solutions were dried over anhydrous magnesium sulphate; evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 Pascals; 4.5-30
15 mm Hg) with a bath temperature of up to 60°C;
- (iii) chromatography unless otherwise stated means flash chromatography on silica gel; thin layer chromatography (TLC) was carried out on silica gel plates; where a "Bond Elut" column is referred to, this means a column containing 10g or 20g of silica of 40 micron particle size, the silica being contained in a 60ml disposable syringe and supported by a porous disc,
20 obtained from Varian, Harbor City, California, USA under the name "Mega Bond Elut SI"
- (iv) in general, the course of reactions was followed by TLC and reaction times are given for illustration only;
- (v) yields are given for illustration only and are not necessarily those which can be obtained by diligent process development; preparations were repeated if more material was required;
- 25 (vi) when given, ¹H NMR data is quoted and is in the form of delta values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, determined at 300 MHz using perdeuterio chloroform (CDCl₃) as the solvent unless otherwise stated; coupling constants (J) are given in Hz;
- (vii) chemical symbols have their usual meanings; SI units and symbols are used;
- 30 (viii) solvent ratios are given in percentage by volume;
- (ix) mass spectra (MS) were run with an electron energy of 70 electron volts in the chemical

ionisation (CI) mode using a direct exposure probe; where indicated ionisation was effected by electron impact (EI) or fast atom bombardment (FAB); where values for m/z are given, generally only ions which indicate the parent mass are reported, and unless otherwise stated the mass ion quoted is the negative mass ion - (M-H)⁻; and

5 (x) the following abbreviations are used:

DMSO	dimethyl sulphoxide;
DMF	<i>N</i> -dimethylformamide;
DCM	dichloromethane; and
EtOAc	ethyl acetate.

10 (xi) where (R) or (S) stereochemistry is quoted at the beginning of a name, unless further clarified, it is to be understood that the indicated stereochemistry refers to the -C(O)-C*(OH)(R¹)(R²) centre as depicted in formula (I) (where C* is the chiral carbon).

Example 1

15 R-[4-(4-Fluorobenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperidine]

A solution of S-3,3,3-trifluoro-2-trimethylsilyoxy-2-methylpropanoyl chloride (*J. Med. Chem.*, 1999, 42, 2741-2746) (293 mg, 1.2 mmol) in ethyl acetate (5 ml) was added to a stirred mixture of 4-(4-fluorobenzoyl)piperidine (207 mg, 1.0 mmol) and triethylamine (0.21 ml, 1.5 mmol) in ethyl acetate (25 ml). The resultant mixture was stirred at ambient

20 temperature for 2 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was dissolved in methanol (10 ml), treated with 1M hydrochloric acid (2ml) and the mixture stirred at ambient temperature for 1 hour. The methanol was evaporated, the aqueous layer was extracted with ethyl acetate (2x25ml), the ethyl acetate extracts were washed with saturated sodium hydrogen carbonate

25 solution, brine, dried and evaporated. The residue was chromatographed on silica with 10% ethyl acetate / dichloromethane as eluent to give a solid which was recrystallized from ethyl acetate / hexane to give the title compound as a solid (104 mg, 0.3 mmol); M pt, 78-79°C; NMR: 1.7 (s, 3H), 1.8-2.0 (m, 4H), 3.2 (dd, 2H), 3.5 (m, 1H), 4.4 (dd, 2H), 5.25 (s, 1H), 7.15 (dd, 2H) and 8.0 (dd, 2H); MS: 346.

Example 2**R-[2-(S)-Methyl-5-(R)-methyl-4-ethanesulphonyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

A solution of ethane sulphonyl chloride (91 mg, 0.707 mmol) in ethyl acetate (10 ml) was added to a stirred mixture of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (150 mg, 0.59 mmol) and triethylamine (0.125 ml, 0.886 mmol) in ethyl acetate (15 ml). The resultant mixture was stirred at ambient temperature for 4 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was recrystallized from ethyl acetate / hexane to give the title compound as a solid (97 mg, 0.28 mmol); M pt, 142-144°C; NMR: 1.15 (d, 3H), 1.3 (m, 6H), 1.65 (s, 3H), 2.9 (m, 2H), 3.3 (s, 1H), 3.35 (q, 2H), 4.05 (s, 1H), 4.15 (s, 1H), 4.4 (s, 1H), and 4.7 (s, 1H); MS: 345.

Example 3**R-[2-(S)-Methyl-5-(R)-methyl-4-(thien-2-ylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

A solution of 2-thiophene sulphonyl chloride (129 mg, 0.707mmol) in ethyl acetate (10 ml) was added to a stirred mixture of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (150 mg, 0.59mmol) and triethylamine (0.125 ml, 0.886mmol) in ethyl acetate (15 ml). The resultant mixture was stirred at ambient temperature for 4 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was recrystallized from ethyl acetate / hexane to give the title compound as a solid (71 mg, 0.18mmol); M pt, 116-117°C; NMR: 0.95 (d, 3H), 1.25 (dd, 3H), 1.65 (s, 3H), 3.2 (dd, 1H), 3.3 (s, 1H), 3.5 (dd, 1H), 4.05 (s, 1H), 4.15 (s, 1H), 4.3 (s, 1H), 4.75 (s, 1H), 7.0 (dd, 1H), and 7.5 (dd, 2H); MS: 399.

Example 4**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-carboxyphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

A solution of 4-chlorosulphonyl benzoic acid (265 mg, 1.2 mmol) in ethyl acetate (10 ml) was added to a stirred mixture of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (254 mg, 1.0mmol) and triethylamine (0.31 ml, 2.2 mmol) in ethyl acetate (15 ml). The resultant mixture was stirred at ambient temperature for 2 hours, washed with 1M hydrochloric acid, brine, dried and evaporated. The residue was recrystallized from ethyl acetate / hexane to give the title compound as a solid (410 mg, 0.936 mmol); Mp, 194-196°C; NMR: 0.9 (d, 3H), 1.2 (dd, 3H), 1.55 (s, 3H), 3.0 (dd, 1H), 3.15 (dd, 1H), 3.4 (s, 1H), 4.1 (m, 2H), 4.6 (s, 1H), 5.15 (s, 1H), 7.75 (d, 2H), and 8.05 (d, 2H); MS: 437.

Example 5**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-dimethylcarbamoylphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

Oxalyl chloride (0.08 ml, 0.88 mmol) was added to a solution of R-[2-(S)-Methyl-5-(R)-methyl-4-(4-carboxyphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (320 mg, 0.73 mmol) (Example 4) in dichloromethane (25 ml). The resultant mixture was stirred at ambient temperature for 4 hours, evaporated to dryness, the residue treated with 40% aqueous dimethylamine (0.82 ml, 7.3 mmol) and the resultant mixture was stirred at ambient temperature overnight. The mixture was treated with ethyl acetate (25 ml), washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was chromatographed on silica with 50% ethyl acetate in dichloromethane as eluent to give a solid which was recrystallized from ethyl acetate / hexane to give the title compound as a solid (26 mg, 0.056 mmol); M pt, 86-87°C; NMR: 0.95 (d, 3H), 1.25 (dd, 3H), 1.65 (s, 3H), 2.95 (s, 3H), 3.1 (s, 3H), 3.25 (dd, 1H), 3.35 (dd, 1H), 3.5 (dd, 1H), 4.2 (m, 2H), 4.5 (s, 1H), 4.8 (s, 1H), 7.5 (d, 2H), and 7.85 (d, 2H); MS: 464.

Example 6**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-fluorophenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

A solution of 4-fluorobenzene sulphonyl chloride (234 mg, 1.2mmol) in ethyl acetate (10 ml) was added to a stirred mixture of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (254 mg, 1.0 mmol) and triethylamine (0.17 ml, 1.2 mmol) in ethyl acetate (15 ml). The resultant mixture was stirred at ambient temperature for 2 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was recrystallized from ethyl acetate / hexane to give the title compound as a solid (230 mg, 0.56 mmol); Mp, 174-175°C; NMR: 0.95 (d, 3H), 1.25 (d, 3H), 1.75 (s, 3H), 3.25 (dd, 1H), 3.35 (d, 1H), 3.5 (dd, 1H), 4.15 (m, 2H), 4.3 (s, 1H), 4.75 (s, 1H), 7.2 (d, 2H), and 7.8 (d, 2H); MS: 411.

Example 7**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-fluorobenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

A solution of 4-fluorobenzoyl chloride (190 mg, 1.2 mmol) in ethyl acetate (10 ml) was added to a stirred mixture of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (254 mg, 1.0mmol) and triethylamine (0.17 ml, 1.2 mmol) in ethyl acetate (15 ml). The resultant mixture was stirred at ambient temperature for 2 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was chromatographed on silica with 20% ethyl acetate in dichloromethane as eluent to give the title compound as a solid which was recrystallized from ethyl acetate / hexane (290 mg, 0.77 mmol); Mp, 177-178°C; NMR: 1.25 (d, 6H), 1.75 (s, 3H), 3.4 (m, 3H), 4.0 (m, 1H), 4.2 (m, 1H), 4.5 (s, 1H), 4.75 (s, 1H), 7.2 (d, 2H), and 7.8 (d, 2H); MS: 375.

Example 8**R-[5-(4-methylsulphanylphenylsulphanyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)indoline]**

Oxalyl chloride (0.35 ml, 4.0 mmol) was added to a stirred suspension of

- 5 (R)-(+)-2-hydroxy-2-methyl-3,3,3-trifluoropropanoic acid (0.58 g, 3.7 mmol) (Method A) in dichloromethane (10 ml) containing DMF (1 drop). The mixture was stirred at ambient temperature for 18 hours and was then added to a solution of 5-(4-methylsulphanylphenylsulphanyl)indoline (1.03 g) (Method B) and 2,6-diphenylpyridine (0.84 g, 3.64 mmol) in dichloromethane (20 ml). The mixture was stirred for 16 hours, volatile material was
- 10 removed by evaporation, and the residue was purified by flash column chromatography on silica gel eluting with 10-20% ethyl acetate / *iso*-hexane to give the title compound (0.935 g, 2.3 mmol) as a solid; NMR: 1.80 (s, 3H), 2.50 (s, 3H), 3.11-3.19 (m, 2H), 4.19-4.29 (m, 1H), 4.38-4.47 (m, 1H), 4.86 (s, 1H), 7.19-7.27 (m, 6H), 8.20 (d, 1H); MS (+ve ESP): 414 (M+H)⁺.

15 **Example 9**

R-[5-(4-methylsulphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)indoline]

Hydrogen peroxide (5 ml of a 30 wt. % solution in water) was added to a solution of R-[5-(4-methylsulphanylphenylsulphanyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)

- 20 indoline] (0.53 g, 1.3 mmol) (Example 8) in glacial acetic acid (10 ml) and the mixture was heated at 95°C for 1 hour. The reaction mixture was allowed to cool to room temperature, ethyl acetate (150 ml) was added and the mixture was washed with saturated aqueous sodium hydrogen carbonate solution (2 x 150 ml) and brine (150 ml) and dried. Volatile material was removed by evaporation and the residue was purified on a silica gel Mega Bond Elut column
- 25 eluting with 0-60% ethyl acetate / *iso*-hexane to give the title compound (0.42 g, 0.88 mmol) as a white solid; NMR (DMSO): 1.61 (s, 3H), 3.16-3.22 (m, 2H), 3.29 (s, 3H), 4.34-4.55 (m, 2H), 7.33 (s, 1H), 7.86-7.90 (m, 2H), 8.12-8.25 (m, 5H); MS: 476.

Example 10**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-dimethylcarbamoylbenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

To a stirred solution of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (400mg, 1.57mmol) and triethylamine (0.4ml, 2.84mmol) in EtOAc (25ml) was added a solution of 4-dimethylcarbamoylbenzoyl chloride (350mg, 1.66mmol) in EtOAc (25ml). The resultant mixture was stirred at ambient temperature for 24 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was chromatographed on silica with EtOAc as eluent to give the title compound as a solid (120mg, 0.28mmol). NMR: 1.1-1.3 (m, 6H), 1.6 (s, 3H), 2.9 (s, 3H), 3.1 (s, 3H), 3.25 (m, 1H), 3.4 (m, 1H), 4.25 (s, 2H), 4.65 (s, 1H), 4.9 (s, 1H), 5.2 (s, 1H) and 7.3-7.4 (dd, 4H); m/z 428.

Example 11**R-{2-(S)-Methyl-5-(R)-methyl-4-[4-(2-hydroxyethylamino)phenylsulphonyl]-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine}**

To a solution of R-[2-(S)-methyl-5-(R)-methyl-4-(4-fluorophenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (Example 6, 350mg, 0.85mmol) in 1-methyl-2-pyrrolidone (2ml) was added ethanolamine (114mg, 1.87mmol) and the mixture heated at 120°C for 24 hours. The mixture was cooled to ambient temperature, poured onto saturated ammonium chloride solution (30ml), the mixture extracted with diethyl ether (2x30ml), the combined ether extracts washed with brine, dried and evaporated. The residue was chromatographed on silica with EtOAc as eluent to give the title compound as a solid (143mg, 0.32mmol). NMR: 0.95 (d, 3H), 1.55 (d, 3H), 1.6 (s, 3H), 3.1 (m, 2H), 3.3 (dt, 2H), 3.4 (d, 1H), 3.8 (dt, 2H), 4.2 (s, 1H), 4.6 (m, 2H), 6.6 (d, 2H), and 7.5 (d, 2H); m/z 411.

Example 12**R-[2-(S)-Methyl-5-(R)-methyl-4-(4-cyanophenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]**

To a stirred solution of R-[2-(S)-methyl-5-(R)-methyl-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine] (*J. Med. Chem.*, 1999, 42, 2741-2746) (254mg, 1.0mmol) and

triethylamine (0.17ml, 1.2mmol) in EtOAc (15ml) was added a solution of 4-cyanobenzene sulphonyl chloride (220mg, 1.1mmol) in EtOAc (10ml). The resultant mixture was stirred at ambient temperature for 2 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was recrystallized from EtOAc / hexane to give the title compound as a solid (280 mg, 0.56mmol). Mp: 186-187°C; NMR: 0.95 (d, 3H), 1.25 (d, 3H), 1.75 (s, 3H), 3.25 (dd, 1H), 3.35 (d, 1H), 3.5 (dd, 1H), 4.2 (m, 3H), 4.75 (s, 1H), 7.8 (d, 2H), and 7.9 (d, 2H); m/z 418.

Examples 13-19

The procedure described in Example 12 was repeated using the appropriate acid chloride or sulphonyl chloride to replace the 4-cyanobenzene sulphonyl chloride to obtain the compounds described below.

No	Example	MS
13	R-[2-(S)-Methyl-5-(R)-methyl-4-(2-chloro4-cyanophenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	452
14	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-acetylphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	435
15	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-methoxyphenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	423
16	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-bromophenylsulphonyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	471
17	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-methoxybenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	387
18	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-dimethylaminobenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	330
19	R-[2-(S)-Methyl-5-(R)-methyl-4-(4-bromobenzoyl)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperazine]	435

Example 20**R-[4-(4-Cyanobenzoylamino)-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperidine]**

To a solution of R-[4-amino-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl) piperidine] (Example 21, 170mg, 0.71mmol) and triethylamine (0.12ml, 0.86mmol) in EtOAc (15ml) was added a solution of 4-cyanobenzoylchloride (130mg, 0.78mmol) in EtOAc (10ml). The resultant mixture was stirred at ambient temperature for 4 hours, washed with 1M hydrochloric acid, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was chromatographed on silica with 50% EtOAc / DCM as eluent to give the title compound as a solid (97mg, 0.26mmol). NMR: 1.45 (m, 2H), 1.65 (s, 3H), 2.1 (m, 2H), 3.05 (m, 2H), 4.2 (m, 1H), 4.4-4.6 (m, 2H), 5.1 (s, 1H), 6.2 (d, 1H), 7.65 (d, 2H) and 7.8 (d, 2H); m/z 368.

Example 21**15 R-[4-Amino-1-(3,3,3-trifluoro-2-hydroxy-2-methylpropionyl)piperidine]**

To a solution of 4-*t*-butyloxycarbonylaminopiperidine (Method C, 8.0g, 40.0mmol) and triethylamine (8.5ml, 60mmol) in dichloromethane (200ml) was added a solution of S-3,3,3-trifluoro-2-trimethylsilyloxy-2-methylpropanoyl chloride (*J. Med. Chem.*, 1999, 42, 2741-2746) (10.1g, 41.3mmol) in dichloromethane (50ml). The resultant mixture was stirred at ambient temperature overnight, the dichloromethane evaporated, the residue taken up in EtOAc (150ml), washed with saturated citric acid solution, saturated sodium hydrogen carbonate solution, brine, dried and evaporated. The residue was dissolved in methanol (25ml), treated with saturated hydrogen chloride in methanol (100ml), stirred at ambient temperature overnight and evaporated to dryness. The residue was recrystallized from methanol / EtOAc to give the title compound as the hydrochloride salt (7.5g, 27.2mmol). Mpt 245-246°C; NMR: 1.45 (m, 2H), 1.5 (s, 3H), 1.95 (m, 2H), 3.25 (m, 1H), 4.4 (m, 2H), 4.65 (m, 2H), 7.15 (s, 1H) and 8.2 (s, 3H); m/z (+ve ESP) 241 (M+H).

Preparation of Starting Materials

30 The starting materials for the Examples above are either commercially available or are readily prepared by standard methods from known materials. For example the following

reactions are illustrations but not limitations of the preparation of some of the starting materials used in the above reactions.

Method A

5 (R)-(+)-2-Hydroxy-2-methyl-3,3,3-trifluoropropanoic acid

R/S-2-Hydroxy-2-methyl-3,3,3-trifluoropropanoic acid was resolved according to the resolution method described in European Patent Application No. EP 524781 (described for the preparation of the (S)-(-) acid) except that (1S, 2R)-norephedrine was used in place of (1R, 2S)-norephedrine or (S)-(-)-1-phenylethylamine to yield the title compound, $[\alpha]_D^{20} +18.1^\circ$ (c, 10 8.8 in MeOH); NMR analysis of the acid in the presence of (R)-(+)-1-phenylethylamine gave an enantiomeric purity of >98%. NMR (CDCl₃): 1.27 (s, 3H) for the (R)-enantiomer, 1.21 (s, 3H) for the (S)-enantiomer.

Method B

15 5-(4-Methylsulphanylphenylsulphanyl)indoline

Tetrakis(triphenylphosphine)palladium(0) (0.99 g, 0.86 mmol) was added to a stirred solution of 5-bromoindoline (4.3 g, 21.7 mmol), 4-methylthiophenylthiol (3.4 g, 21.7 mmol) and sodium methoxide (2.4 g, 72.7 mmol) in anhydrous *n*-butanol (90 ml). The reaction mixture was heated under reflux with stirring for 6 hours then cooled and partitioned between 20 ethyl acetate (200 ml) and brine (100 ml). The organic layer was separated, dried and volatile material was removed by evaporation. The residue was purified by flash column chromatography eluting with 0-30% ethyl acetate / *iso*-hexane to give the title compound (4.2 g, 15.3 mmol) as a solid; NMR: 2.43 (s, 3H), 3.03 (t, 2H), 3.6 (t, 2H), 6.58 (d, 1H), 7.05-7.27 (m, 6H); MS (+ve ESP): 274 (M+H)⁺.

25

Method C

4-*t*-Butyloxycarbonylaminopiperidine

1-Benzyl-4-*t*-butyloxycarbonylaminopiperidine (Method D, 5.4g, 18.6mmol) was dissolved in absolute ethanol (100ml) and hydrogenated over 10% Pd / C for 24 hours at 30 ambient temperature. The catalyst was removed by filtration and the filtrates evaporated to give the title compound (3.54g, 17.7mmol) which was used without further purification.

Method D**1-Benzyl-4-*t*-butyloxycarbonylaminopiperidine**

To a stirred solution of 1-benzyl-4-aminopiperidine (3.8g, 20mmol) in water (20ml) and *t*-butanol (20ml) was added a solution of di-*t*-butyldicarbonate (5.24g, 24mmol) in *t*-butanol (20ml). The mixture was heated at 50°C for 2 hours, cooled to ambient temperature, the *t*-butanol evaporated, the aqueous residue extracted with EtOAc (2x50ml), the combined EtOAc extracts washed with brine, dried and evaporated to give the title compound which was used without further purification.

10 Example 22

The following illustrate representative pharmaceutical dosage forms containing the compound of formula (I), or a pharmaceutically acceptable salt or *in vivo* hydrolysable ester thereof (hereafter compound X), for therapeutic or prophylactic use in humans:-

(a): Tablet I	mg/tablet
Compound X	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

(b): Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c): Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d): Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur	488.5
Magnesium stearate	1.5

(e): Injection I	(50 mg/ml)
Compound X	5.0% w/v
1M Sodium hydroxide solution	15.0% v/v
0.1M Hydrochloric acid	(to adjust pH to 7.6)
Polyethylene glycol 400	4.5% w/v
Water for injection	to 100%

(f): Injection II	10 mg/ml
Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1M Sodium hydroxide solution	15.0% v/v
Water for injection	to 100%

(g): Injection III	(1mg/ml,buffered to pH6)
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection	to 100%

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100

101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200